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New 3,4-*seco*-diterpene and coumarin derivative from the leaves of *Trigonostemon flavidus* Gagnep

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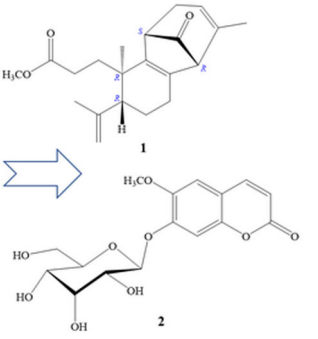
ABSTRACT

Two new compounds named trigoflavidus A (**1**) and trigoflavidus B (**2**), and eight known compounds, trigoflavidone (**3**), heterophypene (**4**), howpene C (**5**), 3,4-*seco*-sonderianol (**6**), trigonochinene C (**7**), fraxidin (**8**), isofraxidin (**9**), and isofraxetin (**10**) were isolated from the leaves of *Trigonostemon flavidus* Gagnep. by various chromatographic methods. Their chemical structures were elucidated via UV, IR, HR-ESI-MS and NMR spectroscopic methods and divided into two groups including six 3,4-*seco*-diterpenes (**1**, **3**-**7**) and four coumarins (**2**, **8**-**10**). Absolute configurations at stereocenters of compound **1** were confirmed by comparison of its CD spectra with those of the TD-DFT calculations. At a concentration of 30 μM, compounds **1**–**10** exhibited weak cytotoxic activity toward LU1, HepG2, MCF7, and SKMel2 human cell lines (cell viability all over 50%).



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1. Introduction

The genus *Trigonostemon* Blume consists approximately 85 species in the family Euphorbiaceae, which are mainly distributed in tropical and subtropical regions of the Asia with 22 species being endemic to Vietnam (Chen et al. 2011; Tagane et al. 2017). Terpenoids, especially diterpenoids, triterpenoids, alkaloids, steroids, flavonoids, lignans, coumarins, and phenolics have been reported from this genus and regarded as the major constituents, displaying diverse biological effects such as insecticidal, antifeedant, antimicrobial, cytotoxic, antitumor, and anti-HIV-1 activities (Chen et al. 2011; Xu and Yue 2014; Xi et al. 2019; Ban et al. 2020). Previous phytochemical investigation on *Trigonostemon flavidus* Gagnep. (known as *Trigonostemon heterophyllus* Merrill) (Li and Gilbert 2008) growing in China resulted in the isolation of diterpenoids (Dong et al. 2011; Tang, He, et al. 2012; Tang, Zhang, et al. 2012). However, up to now, there has not been any research on the chemical constituents as well as biological activity of this plant growing in Vietnam. As a part of our ongoing research on genus *Trigonostemon* (Ban et al. 2020), this paper reports the isolation, structural elucidation of six diterpenes and four coumarins including two new ones isolated from the methanol extract of the *T. flavidus* leaves and their cytotoxic activity toward some cancer cell lines.

2. Results and discussion

Compound **1** was obtained as colorless amorphous powder. Its molecular formula was established as $C_{21}H_{28}O_3$ by the HR-ESI-MS quasi-molecular ion peaks at *m/z* 351.1940 $[M+Na]^+$ (Calcd. for $[C_{21}H_{28}O_3Na]^+$, 351.1931), *m/z* 329.2119 $[M+H]^+$ (Calcd. for $[C_{21}H_{29}O_3]^+$, 329.2111) indicating 8 degrees of unsaturation. The infrared (IR) spectrum of **1** exhibited the presence of carbonyl (1714 cm^{-1}) and C-O-C (1076 cm^{-1}) groups in the molecule. The proton nuclear magnetic resonance spectroscopy (^{1}H NMR) of **1** indicated signals corresponding to three singlet methyl groups at δ_H 1.05, 1.76, and 1.79 (each 3H, s), one methoxy group at δ_H 3.65 (3H, s), one olefinic proton at δ_H 5.19 (1H, broad singlet). The ^{13}C NMR spectrum of compound **1** contained signals corresponding to 21 carbon atoms. Of these, signals at δ_C 209.8 and 174.0 indicated the presence of a ketone and a carboxylate group, respectively. Three double bonds were identified by six olefinic carbon signals at δ_C 114.3 (CH_2), 119.9 (CH), 139.5 (C), 141.2 (C), 145.8 (C × 2), three methyl groups were at δ_C 23.0, 24.1, and 24.5, and a methoxy group was at δ_C 51.6. In the ^{1}H-^{1}H COSY spectrum of **1**, cross peaks between protons H-1 (δ_H 1.72, 1.88) and H-2 (δ_H 1.87, 2.25), H-6 (δ_H 1.70, 1.82) and H-5 (δ_H 2.19)/H-7 (δ_H 2.14, 2.31), and between H-13 (δ_H 5.19) and H-12 (δ_H 2.47, 2.63) were observed indicating the fragments of its molecule. The above evidence suggested that compound **1** was a methoxylated diterpene (Tang, He, et al. 2012; Tang, Zhang, et al. 2012). A comparison of the NMR data of compound **1** with the corresponding data of trigoflavidones A-E, isolated from *T. flavidus* pointed out that this compound is a 3,4-*seco*-diterpenoid (Tang, He, et al. 2012; Tang, Zhang, et al. 2012). However, the NMR data of the C ring of **1** differed from the corresponding of trigoflavidones A-E. These partial assignments together with HR-ESI-MS results required five degree of unsaturation, and therefore the molecule should possess three cyclic patterns. It means that ring C was

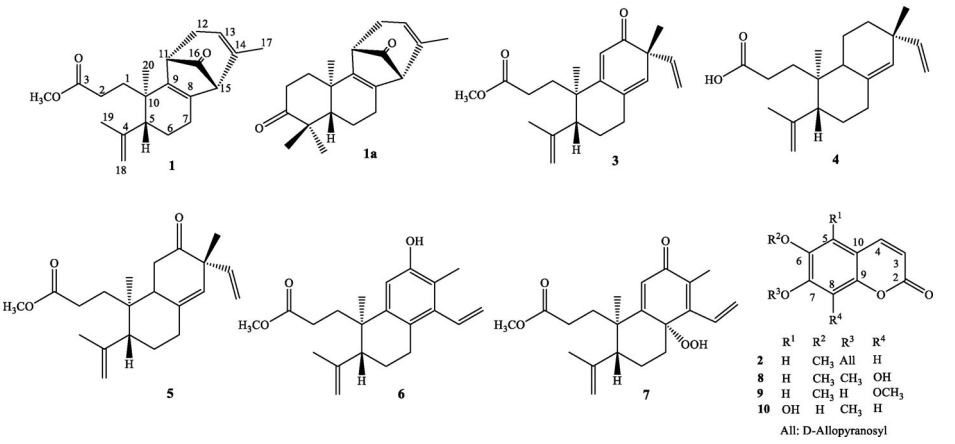

Figure 1. Chemical structures of compounds **1–10** and **1a.**

suggested to become bicyclic patterns as compound **1a**, a Norrish type 1 photodegraded product (Maslovskaya et al. 2011) and suggeted structure of compound **1** was shown in Figure 1. A detailed structural elucidation of compound **1** was further performed using data from HSQC and HMBC experiments. HMBC correlations from H-20 (δ_H 1.05) to C-1 (δ_C 32.1), C-5 (δ_C 46.1), C-9 (δ_C 139.5), C-10 (δ_C 39.8), from H-19 (δ_C 1.76) to C-4 (δ_C 145.8), C-5, C-18 (δ_C 114.3), from H-18 (δ_C 4.67/4.94) to C-5, and C-19 (δ_C 23.0), and from methoxy proton at δ_H 3.65 to C-3 (δ_C 174.0) confirmed the 3,4-*seco*-diterpenoid structure similar to trigoflavidones A-E (Tang, He, et al. 2012; Tang, Zhang, et al. 2012). The bicyclic structure of **1** was further confirmed by HMBC correlations from H-7 (δ_H 2.14/2.31) to C-8 (δ_C 145.8), C-9 (δ_C 139.5), from H-15 (δ_H 2.50) to C-16 (δ_C 209.8), C-9, C-13 (δ_C 119.9), C-14 (δ_H 141.2), C-17 (δ_C 24.1), from H-17 (δ_H 1.79) to C-13, C-14, C-15, and from H-11 (δ_H 2.74) to C-8, C-13, C15, C-16. As for the stereochemistry, the relative configuration of **1** was determined by NOESY experiment. The NOEs cross peaks from H_3-19 (δ_H 1.76) to H_3-20 (δ_H 1.05), H-20 to H-11 (δ_H 2.74) and H_a-12 (δ_H 2.47), and from H-17 (δ_H 1.79) to H-13 (δ_H 5.19) and H-15 (δ_H 2.50) indicated Me-20 α-axially oriented, H-5 β-axially oriented, and H-20, H-11, H-12, H-13, H-15, and H-17 were on the same side as compound **1a** (Maslovskaya et al. 2011). Finally, the absolute configurations of **1** was determined by ECD spectra. Two enantiomers of **1**, (5*R*,10*R*,11*S*,15*R*) and (5*S*,10*S*,11*R*,15*S*), were submitted to TD-DFT calculation their ECD spectra using Gaussian 16 program. Comparison calculated ECD spectra to experimental ECD spectrum of **1** indicated absolute configurations of **1** to be 5*R*,10*R*,11*S*,15*R* (Figure 3). Consequently, the chemical structure of compound **1** was fully established (Figure 1). This is a new 3,4-*seco*-diterpenoid and named as trigoflavidus A.

Compound **2** was obtained as colorless amorphous powder. The HR-ESI-MS analysis of **2** revealed a quasi-molecular ion peaks at *m/z* 355.1023 $[M+H]^+$ (Calcd. for $[C_{16}H_{19}O_9]^+$, 355.1024), *m/z* 389.0634 $[M + {}^{35}Cl]^-$ (Calcd. for $[C_{16}H_{18}O_9{}^{35}Cl]^-$, 389.0645), and *m/z* 391.0618 $[M + {}^{37}Cl]^-$ (Calcd. for $[C_{16}H_{18}O_9{}^{37}Cl]^-$, 391.0681), indicating a molecular formula of $C_{16}H_{18}O_9$. The IR spectrum of **2** exhibited the presence of hydroxyl (3401 cm^{-1}), carbonyl (1711 cm^{-1}) and C-O-C (1073 cm^{-1}) groups in the molecule. The ^{1}H-NMR spectrum of **2** exhibited four olefinic proton signals including two singlets at δ_H 7.22 and 7.19, and two doublets at δ_H 7.92 and 6.32 ($J = 9.5$ Hz), one

methoxy group at δ_H (3.92), one anomeric proton at δ_H 5.40 (d, $J = 8.0$ Hz), and six oxygenated methine protons at δ_H 3.65-4.19. The ^{13}C-NMR spectrum of **2** indicated the presence of nine carbon atoms at δ_C 105.1, 110.8, 114.4, 145.7, 148.3, 150.7, and 163.6, assigning for a coumarin molecule, one methoxy group at δ_C 57.1, and one sugar at δ_C 100.3, 76.0, 73.0, 71.9, 68.5, and 62.7. The assignments of protons belonging to carbons were taken by HSQC spectrum. The above data suggested that compound **2** was a coumarin glycoside having a methoxy group. The NMR data of **2** were similar to the corresponding data of scopolin (7-*O*-β-D-glucopyranosyl-scopoletin) (Kuroyanagi et al. 1986) except for the sugar data, suggesting that compound **2** was a scopoletin glycosyl derivative. For the sugar moiety, a set of carbon chemical shifts of **2** (from C-1′ to C-6′: δ_C 100.3, 71.9, 73.0, 68.5, 76.0, 62.7) were found to very similar to the corresponding data of the allose unit of charantoside A (Nhiem et al. 2010). Detail analysis of the proton and carbon chemical shifts as well as the ^{1}H-^{1}H coupling constants of the sugar unit of **1** matched perfectly with those of the allose sugar (Nhiem et al. 2010). Proton appeared at δ_H 4.19 as a triplet with a small coupling constant ($J = 3.0$ Hz) confirming that this proton was in *equatorial* orientation. In addition, HMBC correlations from proton at δ_H 4.19 to C-1′ (δ_C 100.3), C-2′ (δ_C 71.9), C-4′ (δ_C 68.5), C-5′ (δ_C 76.0) were observed indicating that this signal corresponds to the proton H-3′ of the allose unit. The sugar linkage must be in the β-form as judged from coupling constant ($J = 8.0$ Hz) of the anomeric proton signal at δ_H 5.40. The HMBC correlation from H-1′ (δ_H 5.40) to C-7 (δ_C 152.1) indicated the allose sugar linked to C-7 of the aglycone. Finally, monosaccharide in the sugar residue was confirmed to be D-allose by hydrolysis (Nhiem et al. 2010). Consequently, compound **2** was determined to be 7-*O*-β-D-allopyranosyl-scopoletin, a new compound and named as trigoflavidus B.

The other compounds isolated from *Trigonostemon flavidus* Gagnep. were identified as trigoflavidone (**3**) (Tang, He, et al. 2012), heterophypene (**4**) (Xi et al. 2019), howpene C (**5**) (Ma et al. 2017), 3,4-*seco*-sonderianol (**6**) (Craveiro and Silveira 1982), trigonochinene C (**7**) (Yin et al. 2008), fraxidin (**8**) (Hu et al. 2011), isofraxidin (**9**) (Tsukamoto et al. 1985), and isofraxetin (**10**) (Majnooni et al. 2020) by comparisons of their NMR data with those data reported in the literature.

Compounds **3**-**7** were previously isolated from genus *Trigonostemon* growing in China, however compounds **8**-**10** were first reported from this genus and compounds **3**-**10** were first found from *T. flavidus* growing in Vietnam.

The cytotoxic effect of compounds **1**–**10** was evaluated on four human cancer cell lines including LU1 (lung cancer), HepG2 (liver cancer), MCF7 (breast cancer) and SKMel2 (skin cancer). Each compound was first examined cytotoxic activity at concentration of 30 μM using sulforhodamine B assay (Monks et al. 1991). Because compounds **1**–**10** exhibited weak cytotoxic activities with cell viability percentages all higher than 50% (Supplementary data), they were not subjected for further evaluation on cytotoxic study.

3. Experimental

3.1. General

Optical rotation was measured on a Jasco P2000 polarimeter. CD spectrum was obtained on a Chirascan spectrometer. IR spectra were recorded on a Spectrum Two

FT-IR spectrometer. HR-ESI-MS spectra were acquired on an Agilent 6530 Accurate Mass Q-TOF LC/MS. NMR spectra were recorded on a Bruker 500 MHz spectrometer. Preparative HPLC were run on an Agilent 1100 system including quaternary pump, autosampler, DAD detector, and preparative HPLC column YMC J'sphere ODS-H80 (4 μm, 20 × 250 mm). Isocratic mobile phase with the flow rate of 3 mL/min was used in pre-HPLC. The compounds were monitored at wavelengths of 205, 230, 254 and 280 nm. Flash column chromatography was performed using silica gel, reversed phase C-18, and diainon HP-20 resins as the adsorbent. Thin layer chromatography was carried out on pre-coated silica gel 60 F_{254} and RP-18 F_{254S} plates. The spots were detected by spraying with aqueous solution of H_2SO_4 5% followed by heating with a heat gun.

3.2. Plant material

The leaves of *Trigonostemon flavidus* Gagnep. were collected at Melinh District, Vinh Phuc Province, Vietnam in September 2019 and taxonomically identified by Dr Do Van Hai at the Institute of Ecology and Biological Resources, VAST. Voucher specimen (TNSV-ML-02-NKB) was deposited at the Institute of Marine Biochemistry, VAST.

3.3. Extraction and isolation

Dried powdered leaves of *T. flavidus* (5 kg) was ultrasonically extract with methanol at room temperature for three times (each, 10 L of methanol in 2 h). The solvent was then removed *in vacuo* to give methanol extract. This extract (250 g) was suspended in distilled water (3 L) and successively partitioned with *n*-hexane, dichloromethane, and ethyl acetate to give four soluble fractions including *n*-hexane fraction (10 g), dichloromethane fraction (70 g), ethyl acetate fraction (5 g), and water layer. Dichloromethane and ethyl acetate fractions were combined and separated on a silica gel column, eluting with gradient solvent system of *n*-hexane/acetone (0-100% acetone) to give four fractions DE1- DE4. Fraction DE1 (16 g) was loaded on a silica gel column and eluted with *n*-hexane/ethyl acetate (8/1, v/v) to give five fractions DE1A-DE1E. Fraction DE1C was firstly chromatographed on a reversed phase C18 (RP18) column, eluting with acetone/water (4/1, v/v) and then purified by pre-HPLC using ACN in water (88% in volume) to give compound **5** (27 mg, t_R 45.0 min). Fraction DE1D was separated on a RP18 column, eluting with methanol/water (9/2, v/v) to give five fractions DE1D1- DE1D5. Fraction DE1D2 was purified by pre-HPLC using ACN in water (75% in volume) to give compound **7** (6.5 mg, t_R 35.4 min). Fraction DE1D5 was purified by pre-HPLC using ACN in water (80% in volume) to give compound **1** (7.5 mg, t_R 62.2 min). Fraction DE1C was purified on a RP18 column, eluting with methanol/water (9/2, v/v) to obtain compounds **3** (27 mg), **4** (51 mg), and **6** (38 mg). Fraction DE3 (9 g) was separated on a silica gel column, eluting with dichloromethane/methanol (40/1, v/v) to give five fractions DE3A-DE3E. Fraction DE3A was continuously chromatographed on a RP18 column, eluting with methanol/water (2/1, v/v) to give four fractions DE3A1-DE3A4. Fraction DE3A1 was purified by pre-HPLC using ACN in water (25% in volume) to give compound **9** (12 mg, t_R 43.2 min). Fraction DE3A3 was purified by

pre-HPLC using ACN in water (45% in volume) to give compound **10** (12 mg, t_R 33.6 min). Fraction DE3C was first separated on a RP18 column, eluting with methanol/water (1/1, v/v) and then purified by pre-HPLC using ACN in water (35% in volume) to give compound **8** (6 mg, t_R 46.5 min). Water layer was poured on a diaion (HP-20) column, washed with water, and then eluted with methanol/water (1/1 and 1/0, v/v, step wise) to give two fractions W1 and W2. Fraction W1 was first chromatographed on a RP18 column, eluting with methanol/water (2/3, v/v) and then purified by pre-HPLC using ACN in water (18% in volume) to give compound **2** (7 mg, t_R 31.4 min).

*3.3.1. Trigoflavidus A (**1**):* Colorless amorphous powder, $[\alpha]_D^{25}$: − 37.8° (*c* 0.1, MeOH); UV (MeOH) λ_{max} 235 nm; IR (KBr): ν_{max} 2,92,71,71,41,076 cm^{-1}; HR-ESI-MS *m/z* 351.1940 $[M+Na]^+$ (Calcd. for $[C_{21}H_{28}O_3Na]^+$, 351.1931), *m/z* 329.2119 $[M+H]^+$ (Calcd. for $[C_{21}H_{29}O_3]^+$, 329.2111). CD (MeOD) $mdeg_{(\lambda)}$: $-14.8_{(221)}$, $-15.2_{(290)}$; ^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 1.72 (1H, m, H_a-1), 1.88 (1H, m, H_b-1), 1.87 (1H, m, H_a-2), 2.25 (1H, m, H_b-2), 2.19 (1H, dd, J = 12.0, 3.0 Hz, H-5), 1.70 (1H, m, H_a-6), 1.82 (1H, m, H_b-6), 2.14 (1H, m, H_a-7), 2.31 (1H, m, H_b-7), 2.47 (1H, d, J = 5.0 Hz, H-11), 2.47 (1H, m, H_a-12), 2.63 (1H, m, H_b-12), 5.19 (1H, brs, H-13), 2.50 (1H, s, H-15), 1.79 (3H, s, H-17), 4.67 (1H, s, H_a-18), 4.94 (1H, s, H_b-18), 1.76 (3H, s, H-19), 1.05 (3H, s, H-20), 3.65 (3H, s, OCH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz), δ (ppm): 32.1 (C-1), 29.2 (C-2), 174.0 (C-3), 145.8 (C-4), 46.1 (C-5), 24.7 (C-6), 25.5 (C-7), 145.8 (C-8), 139.5 (C-9), 39.8 (C-10), 47.1 (C-11), 32.7 (C-12), 119.9 (C-13), 141.2 (C-14), 55.8 (C-15), 209.8 (C-16), 24.1 (C-17), 114.3 (C-18), 23.0 (C-19), 24.5 (C-20), 51.6 (OCH_3).

3.3.2. Trigoflavidus B (2)

Colorless amorphous powder, $[\alpha]_D^{25}$: + 58.2° (*c* 0.1, MeOH); UV (MeOH) λ_{max} 2,04,235 nm; IR (KBr): ν_{max} 3,40,12,93,41,71,11,070 cm^{-1}; HR-ESI-MS *m/z* 355.1023 $[M+H]^+$ (Calcd. for $[C_{16}H_{19}O_9]^+$, 355.1024), *m/z* 389.0634 $[M+^{35}Cl]^-$ (Calcd. for $[C_{16}H_{18}O_9{}^{35}Cl]^-$, 389.0645), *m/z* 391.0618 $[M+^{37}Cl]^-$ (Calcd. for $[C_{16}H_{18}O_9{}^{37}Cl]^-$, 391.0681). ^{1}H-NMR ($CD_3OD$, 500 MHz), δ (ppm): 6.32 (1H, d, *J* = 9.5 Hz, H-3), 7.92 (1H, d, *J* = 9.5 Hz, H-4), 7.22, (1H, s, H-5), 7.19 (1H, s, H-8), 3.92 (3H, s, OCH_3), **All**: 5.40 (1H, d, *J* = 8.0 Hz, H-1′), 3.71 (1H, dd, *J* = 8.0, 3.0 Hz, H-2′), 4.19 (1H, dd, *J* = 3.0, 3.0 Hz, H-3′), 3.65 (1H, dd, *J* = 9.0, 3.0 Hz, H-4′), 3.93 (1H, m, H-5′), 3.70 (1H, dd, *J* = 12.0, 5.0 Hz, H_a-6′), 3.91 (1H, dd, *J* = 12.0, 2.5 Hz, H_b-6′). ^{13}C-NMR (CD_3OD, 125 MHz), δ (ppm): 163.6 (C-2), 114.4 (C-3), 145.7 (C-4), 110.8 (C-5), 148.3 (C-6), 152.1 (C-7), 105.1 (C-8), 150.7 (C-9), 114.5 (C-10), 57.1 (OCH_3), **All**: 100.3 (C-1′), 71.9 C-2′), 73.0 (C-3′), 68.5 (C-4′), 76.0 (C-5′), 62.7 (C-6′).

4. Conclusions

Six 3,4-*seco*-diterpenes and four coumarin derivatives were isolated from the leaves of *T. flavidus*. Among them one 3,4-*seco*-diterpene, trigoflavidus A (**1**), and one coumarin derivative, trigoflavidus B (**2**), are previously undescribed. Their chemical structures were identified by extensive analysis of HR-ESI-MS and NMR spectral data. Additionally, absolute configuration of compound **1** was determined by comparison its experimental ECD spectrum with those of TD-DFT calculations ECD spectra. Compounds **1**-**10** showed weak cytotoxic effect on LU1, HepG2, MCF7, and SKMel2

human cell lines. At concentration of 30 μM, cell viability percentages were all higher than 50% in the presence of compounds **1**-**10.**

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Ban NK, Truong LH, Tiep TV, Yen DTH, Doan VV, Nhiem NX, Seo Y, Namkung W, Kim SH, Tai BH, et al. 2020. Four new sucrose diesters of substituted truxinic acids from Trigonostemon honbaensis with their anoctamin-1 inhibitory activity. Bioorg Chem. 102:104058.

Chen YG, Wu JC, Chen GY, Han CR, Song XP. 2011. Chemical constituents of plants from the genus *Trigonostemon*. Chem Biodivers. 8(11):1958–1967.

Craveiro AA, Silveira ER. 1982. Two cleistanthane type diterpenes from *Croton sonderianus*. Phytochemistry. 21(10):2571–2574.

Dong SH, Liu HB, Xu CH, Ding J, Yue JM. 2011. Constituents of *Trigonostemon heterophyllus*. J Nat Prod. 74(12):2576–2581.

Hu HB, Zheng XD, Jian YF, Liu JX, Zhu JH. 2011. Constituents of the root of *Anemone tomentosa*. Arch Pharm Res. 34(7):1097–1105.

Kuroyanagi M, Shiotsu M, Ebihara T, Kawai H, Ueno A, Fukushima S. 1986. Chemical studies on *Viburnum awabuki* K.KOCH. Chem Pharm Bull. 34(10):4012–4017.

Li BT, Gilbert MG. 2008. Flora of China. Science: Beijing. 11:272–274.

Ma J, Yang X, Wang P, Dong B, Su G, Tuerhong M, Jin DQ, Xu J, Lee D, Ohizumi Y, et al. 2017. Phytochemicals with NO inhibitory effects and interactions with iNOS protein from *Trigonostemon howii*. Bioorg Chem. 75:71–77.

Majnooni MB, Fakhri S, Shokoohinia Y, Mojarrab M, Kazemi AS, Farzaei MH. 2020. Isofraxidin: synthesis, biosynthesis, isolation, pharmacokinetic and pharmacological properties. Molecules. 25(9):2040.

Maslovskaya LA, Savchenko AI, Gordon VA, Reddell PW, Pierce CJ, Parsons PG, Williams CM. 2011. Isolation and confirmation of the proposed cleistanthol biogentic link from *Croton insularis*. Org Lett. 13(5):1032–1035.

Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigro-Wolff A. 1991. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. J Natl Cancer Inst. 83(11):757–766.

Nhiem NX, Kiem PV, Minh CV, Ban NK, Cuong NX, Tung NH, Ha LM, Ha DT, Tai BH, Quang TH, et al. 2010. alpha-Glucosidase inhibition properties of cucurbitane-type triterpene glycosides from the fruits of Momordica charantia. Chem Pharm Bull (Tokyo)). 58(5):720–724.

Tagane S, Yahara T, Dang VS, Toyama H, Tran H. 2017. (Euphorbiaceae), a new species from Mt. Hon Ba, Southern Vietnam. Acta Phytotax Geobot. 68(1):39–44.

Tang GH, He HP, Gu YC, Di YT, Wang YH, Li SF, Li SL, Zhang Y, Hao XJ. 2012. 3,4-*seco*-diterpenoids from *Trigonostemon flavidus*. Tetrahedron. 68(47):9679–9684.

Tang GH, Zhang Y, Gu YC, Li SF, Di YT, Wang YH, Yang CX, Zuo GY, Li SL, He HP, et al. 2012. Trigoflavidols A-C, degraded diterpenoids with antimicrobial activity, from Trigonostemon flavidus. J Nat Prod. 75(5):996–1000.

Tsukamoto H, Hisada S, Nishibe S. 1985. Coumarins from bark of *Fraxinus japonica* and *F. mandshurica* var. *japonica*. Chem Pharm Bull. 33(9):4069–4073.

Xi Y, An L, Yang X, Song Z, Zhang J, Tuerhong M, Jin DQ, Ohizumi Y, Lee D, Xu J, et al. 2019. NO inhibitory phytochemicals as potential anti-inflammatory agents from the twigs of *Trigonostemon heterophyllus*. Bioorg Chem. 87:417–424.

Xu JB, Yue JM. 2014. Recent studies on the chemical constituents of *Trigonostemon plants*. Org Chem Front. 1(10):1225–1252.

Yin S, Su ZS, Zhou ZW, Dong L, Yue JM. 2008. Antimicrobial diterpenes from *Trigonostemon chinensis*. J Nat Prod. 71(8):1414–1417.